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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>7</sup> : <b>A61L 31/08, 27/28</b>		A1	(11) International Publication Number: <b>WO 00/12146</b>  (43) International Publication Date: <b>9 March 2000 (09.03.00)</b>
			(21) International Application Number: PCT/US99/19633  (22) International Filing Date: 27 August 1999 (27.08.99)
(30) Priority Data: 60/098,040 27 August 1998 (27.08.98) US			(81) Designated States: AU, BR, CA, JP, KR, NZ, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
			<b>Published</b> <i>With international search report.</i>
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(54) Title: GELS AND MULTILAYER SURFACE STRUCTURES FROM BORONIC ACID CONTAINING POLYMERS

## (57) Abstract

Boronic acid containing polymers are used to form bioinert gels and multilayer surface structures. These polymers form cross-linked hydrogels, which are highly swollen in water. The cross-linking can either be chemical or physical. Water soluble polymers containing boronic acid groups, such as phenylboronic acid (PBA), can be physically cross-linked by mixing the polymers in water with other polymers containing hydroxyls or carboxylic acids. Alternatively, surfaces can be treated by stepwise incubation with a solution of the boronic acid containing polymer, followed by incubation with a solution of a diol or carboxylic acid containing polymer. Many successive layers can be generated, increasing the thickness of the formed structure at each step. The bioinert gel or surface coating can be used for passivating the surfaces of medical implants (especially those based on transplanted tissue), or for passivating the surfaces of tissues *in situ*, decreasing the incidence or severity of such pathologic conditions as the formation of post-surgical adhesions, and thrombosis following angioplasty.

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## GELS AND MULTILAYER SURFACE STRUCTURES FROM BORONIC ACID CONTAINING POLYMERS

### Background of the Invention

5 This is generally in the field of polymeric materials for modulation of cell to cell interactions, especially for biomedical applications.

#### Hydrogels as controlled-release carriers

Biodegradable hydrogels can be carriers for biologically active materials such as hormones, enzymes, antibiotics, antineoplastic agents, and 10 cell suspensions. Temporary preservation of functional properties of a carried species, as well as controlled release of the species into local tissues or systemic circulation, are possible. Proper choice of hydrogel macromers can produce membranes with a range of permeability, pore sizes and degradation rates suitable for a variety of applications in surgery, medical 15 diagnosis and treatment.

#### Adhesives and sealers

Polymeric hydrogels have also been used as tissue adhesives and sealants. Fibrin gels have been used extensively in Europe as sealants and adhesives in surgery (Thompson *et al.*, 1988, *Drug Intell. and Clin. Pharm.*, 20 22:946; Gibble *et al.*, 1990, (1990), *Transfusion*, 30(8):741). Synthetic polymers have been explored as adhesives (Lipatova, 1986, *Advances in Polymer Science* 79:65-93), but these materials have generally been associated with local inflammation, cytotoxicity, and poor biocompatibility.

#### Prevention of postoperative adhesions.

25 Formation of post-surgical adhesions involving organs of the peritoneal cavity and the peritoneal wall is a frequent and undesirable result of abdominal surgery. Surgical trauma to the tissue caused by handling and drying results in release of a serosanguinous (proteinaceous) exudate which tends to collect in the pelvic cavity (Holtz, G., 1984). If the exudate is not 30 absorbed or lysed within this period it becomes ingrown with fibroblasts, and subsequent collagen deposition leads to adhesion formation.

Numerous approaches to elimination of adhesion formation have been attempted, with limited success in most cases. Approaches have

included lavage of the peritoneal cavity, administration of pharmacological agents, and the application of barriers to mechanically separate tissues. However, none of these approaches has been cost effective and effective in *in vivo* studies. Solutions of Poloxamer 407 have been used for the treatment 5 of adhesions, with some success. Poloxamer is a copolymer of ethylene oxide and propylene oxide and is soluble in water; the solutions are liquids at room temperature. Steinleitner *et al.* (1991) *Obstetrics and Gynecology*, 77(1):48 and Leach *et al.* (1990) *Am. J. Obstet. Gynecol.*, 162(5):1317, examined Poloxamer solutions in peritoneal adhesion models and observed 10 statistically significant reductions in adhesions; however, they were unable to eliminate adhesions, perhaps because of limited adhesion and retention on the injury site. Oxidized regenerated cellulose has also been used extensively to prevent adhesions and is an approved clinical product, trade-named Interceed TC7. This barrier material has been shown to be somewhat 15 effective in rabbits (Linsky *et al.*, 1987 *J. Reprod. Med.*, 32:17; Diamond *et al.*, 1987 *Microsurgery*, 8:103) and in humans (Interceed (TC7) *Adhesion Barrier Study Group*, 1989). It was shown to be more effective if pretreated with heparin, but was still unable to completely eliminate adhesions (Diamond *et al.*, 1991 " *Fertility and Sterility*, 55(2):389). U.S. Patent No. 20 5,410,016 to Hubbell, et al., describes photopolymerizable biodegradable hydrogels as tissue contacting materials and controlled release carriers. These polymers included a water soluble region flanked by biodegradable linkers, terminated in photopolymerizable groups. Despite promising results in a rabbit model of adhesions, results of clinical trials to prevent adhesions 25 following Cesarean sections were mixed, perhaps due to insufficient polymer thickness of the layers.

It is an object of the present invention to provide polymeric materials which form gels, coatings, and multi-layer structures that are bioinert and therefore useful for a variety of biomedical applications, including 30 prevention of adhesions, as sealants, and for controlled delivery.

It is another object of the present invention to provide means for applying these polymeric materials to form coatings and medical devices.

### Summary of the Invention

Boronic acid containing polymers are used to form bioinert gels and multilayer surface structures. These polymers form crosslinked hydrogels, which are highly swollen in water. The crosslinking can either be chemical or physical. Water soluble polymers containing boronic acid groups, such as phenylboronic acid (PBA), can be physically crosslinked by mixing the polymers in water with other polymers containing hydroxyls or carboxylic acids. Alternatively, surfaces can be treated by stepwise incubation with a solution of the boronic acid containing polymer, followed by incubation with a solution of a diol or carboxylic acid containing polymer. Many successive layers can be generated, increasing the thickness of the formed structure at each step. Treatment of the surface is dependent upon the surface activity of the boronate containing polymer or the diol or carboxylic acid containing polymer, or the use of a priming layer, consisting of a molecule which has an affinity for the surface, as well as an affinity for the boronate containing polymer or the diol or carboxylic acid containing polymer. Priming may not be necessary in the case of binding to a cell or tissue surface, because the boronic acid domains bind to diols present in glycosylated proteins present in the cells.

The bioinert gel or surface coating can be used for passivating the surfaces of medical implants (especially those based on transplanted tissue), or for passivating the surfaces of tissues *in situ*, decreasing the incidence or severity of such pathologic conditions as the formation of post-surgical adhesions, and thrombosis following angioplasty.

25

### Detailed Description of the Invention

Boronic acid polymers are described for use in biomedical applications. In one embodiment, these are used in combination with diol or carboxylic acid containing polymers to form multilayer structures. The polymers and structures can be used for drug delivery, coatings or devices, or modified to alter cell attachment or interaction.

## I. Boronic Acid Containing Compositions

### Boronic Acid Polymers

Phenylboronic acid and its derivatives bind with high affinity to molecules containing vicinal or closely opposed diols or carboxylic acids.

- 5 This property has been previously exploited in biotechnology to produce glucose releasing devices (A. Kikuchi et al., *Anal. Chem.*, 68:823-828, 1996), chromatographic media with affinity for polysaccharides (K. Tsukagoshi et al., *Analytical Sciences*, 13:485-487, 1997), and as agents to interact with cell surfaces to promote cell attachment or receptor clustering
- 10 (T. Aoki et al., *J. Biomat. Sci. Polym. Ed.*, 9:1-14, 1997; T. Ikeya et al, *Reactive & Functional Polymers*, 37:251-261, 1998).

Useful boronates include phenylboronic acid (PBA), 2-carboxyethaneboronic acid, 1,2-dicarboxyethaneboronic acid,  $\beta,\beta'$ -dicarboxyethaneboronate,  $\beta,\gamma$ -dicarboxypropaneboronate, 2-nitro- and 15 4-nitro-3-succinamidobenzene boronic acids, 3-nitro-4-(6-aminohexylamido)phenyl boronic acid, {4-[(hexamethylenetetramine)methyl]phenyl} boronic acid, 4-(N-methyl)carboxamidobenzene boronic acid, 2-{[(4-boronphenyl)methyl]-ethylammonio}ethyl and 20 2-{[(4-boronphenyl)methyl]diethylammonio}-ethyl groups, succinyl-3-aminophenylboronic acid, 6-aminocaproyl-3-aminophenylboronic acid, 3-(N-succinimidoxycarbonyl)aminophenylboronate, p-( $\omega$ -aminoethyl)phenylboronate, p-vinylbenzeneboronate, N-(3-dihydroxyborylphenyl)succinamic acid, N-(4-nitro-3-25 dihydroxyborylphenyl)succinamic acid, O-dimethylaminomethylbenzeneboronic acid, 4-carboxybenzeneboronic acid, 4-(N-octyl)carboxamidobenzeneboronic acid, 3-nitro-4-carboxybenzeneboronic acid, 2-nitro-4-carboxybenzeneboronic acid, 4-bromophenylboronate, p-vinylbenzene boronate, 30 4-( $\omega$ -aminoethyl)phenylboronate, catechol [2-(diethylamino)carbonyl, 4-bromomethyl]phenyl boronate, and 5-vinyl-2-dimethylaminomethylbenzeneboronic acid. These boronate containing

groups differ in terms of pKa, spacer arms, or different coupling options associated with them.

In a preferred embodiment, the boronate group is provided by phenylboronic acid (PBA) which is known to form reversible conjugates

5 with coplanar diols, such as closed ring carbohydrates and polyvinyl alcohol, as well as with acidic ligands such as dicarboxylic acids and  $\alpha$ -hydroxy carboxylic acids. PBA has a strong affinity to many biological surfaces, since the surfaces of cells and extracellular matrix are rich in proteoglycans and other carbohydrate moieties, as well as many acidic moieties. PBA also  
10 has been shown to form reversible complexes with glycoconjugates on endothelial cell membranes (T. Aoki *et al.*, Journal of Biomaterials Science Polymer Edition 7:539-550 (1995)) and lymphocyte membranes (H. Miyazaki *et al.*, Biochemical and Biophysical Research Communications 195:829-836 (1993)).

15 The "working pH" of the PBA moieties in each copolymer can be adjusted by placing amine groups in proximity to the PBA groups or by placing electron withdrawing groups within the PBA moiety itself. A PBA moiety with a nitro group in the ring and a succinamic acid functionality has been synthesized by Singhal *et al.*, Journal of Chromatography 543:17-38  
20 (1991), and could be coupled to amine groups using carbonyldiimidazole or N-hydroxysuccinimide. A PBA moiety that has an internal coordinate bond, making the boron tetrahedral, and that has a bromomethyl group that could be reacted with the thiol of cysteine was synthesized by X.-C. Liu *et al.*, Journal of Chromatography A 687:61-69 (1994). A polymer precursor with  
25 a very low pKa, 5-vinyl-2-dimethylaminomethylbenzeneboronic acid that could be used in creating random copolymers containing PBA was synthesized by G. Wulff, Pure and Applied Chemistry 54:2093-2102 (1982).

Any of a number of water soluble polymers incorporating any of the boronate groups listed above can be used as the boronate containing  
30 polymer. Such polymers are known in the art. Examples include poly(ethylene glycol), poly(ethylene oxide), poly(vinyl alcohol), poly(vinylpyrrolidone), poly(ethyloxazoline), poly(ethylene oxide)-co-

poly(propyleneoxide) block copolymers such as polypropylene oxide-polyethylene oxide block copolymers, polysaccharides or carbohydrates such as hyaluronic acid, dextran, heparan sulfate, chondroitin sulfate, heparin, or alginate, and proteins such as gelatin, collagen, albumin, or ovalbumin. The 5 term "at least substantially water soluble" is indicative that the solubility should be at least about 5 g/100 ml of aqueous solution.

*Synthesis of Boronate Containing Polymers*

Polymers comprising phenylboronic acid moieties can be synthesized, for example, by reacting aminophenylboronic acid with acryloyl 10 chloride (D. Shino et al., *J. Biomater. Sci Polym. Ed.*, 7:697-701, 1996), followed by free-radical polymerization with acrylamide to produce poly(acrylamide-co-acrylamidophenylboronic acid).

*Diol or Carboxylic Acid Containing Polymer*

The diol or carboxylic acid containing polymer can be, but is not 15 limited to, dextran, mannan, polysialic acid, polyvinyl alcohol, hyaluronic acid, polyacrylic acid (or a derivative thereof such as methacrylic acid).

*Synthesis of Copolymers*

A copolymer of poly(acrylamide-co-acrylamidophenylboronic acid) and dextran can be synthesized by dissolving the poly(acrylamide-co- 20 acrylamidophenylboronic acid) in buffered saline, and mixing the solution with dextran to form a gel. Phenylboronic acid does not bind to diols at a low pH, thus the pH of the solution could initially be at pH 5, then the pH could be raised to initiate gelation. The gelation could be induced to occur inside the body, for use in the prevention of post-surgical adhesions, by 25 providing a barrier between tissues.

*Polymer Modifications*

The boronic acid containing polymers can have a number of other functionalities within the polymer chain, which can enhance such properties as water solubility, biointeractivity, or charge. The diol or carboxylic acid 30 containing polymers can be synthesized or selected so as to maximize biointeractivity, or can be synthesized or selected to contain other functionalities that enhance such properties as water solubility, biointeractivity, or charge.

Additional polymeric components, domains, linking groups, and bioactive, prophylactic, or diagnostic materials can be added to either of the polymers to modify their properties.

*Linking Groups or Polymers*

5 Examples of additional polymeric components for attachment of linking groups or bioactive, prophylactic, or diagnostic materials include PEG, polyacrylic acid, poly-N-vinyl pyrrolidone, hyaluronic acid, and other polysaccharides. Other domains that can be incorporated into the boronic or diol or carboxylic acid containing polymers include bioadhesive molecules,  
10 domains which convert from a binding domain to a nonbinding domain *in vivo*, and domains which convert from a nonbinding domain to a binding domain *in vivo*, as described in U.S. Patent No. 5,410,016 to Hubbell et al. Examples of linking groups include biodegradable linkages, such as anhydride, ester, amide, and carbonate linkages.

15 *Materials modifying Properties of the Polymeric Materials*

The domains and/or linkages can be used to impart properties to the polymeric material. For example, domains may be incorporated into the polymer so that it selectively adheres to particular types of cells or molecules or is selectively degraded by enzymatic or nonenzymatic means.

20 Degradation of the compositions can be controlled by the incorporation of sites that are degradable either chemically or enzymatically, providing a mechanism for the removal of the structure. The domains may consist of another polymer, for example, a biodegradable polymer such as a polyanhydride, polyhydroxy acid or polycarbonate, which makes the  
25 polymeric material biodegradable. Photopolymerizable substituents, including acrylates, diacrylates, oligoacrylates, dimethacrylates, or oligomethacrylates, and other biologically acceptable photopolymerizable groups, can also be coupled to the polymeric materials. These can be used to further polymerize the polymer once it is in contact with tissue or other  
30 surfaces, which can result in improved adherence to the surface.

Monomers or functional groups to be incorporated, as well as methods for incorporation, are known to those skilled in the art. The time

required for a polymer to degrade can be tailored by selecting appropriate monomers. Differences in crystallinity also alter degradation rates. Due to the relatively hydrophobic nature of these polymers, actual mass loss only begins when the oligomeric fragments are small enough to be water soluble.

5 Hence, initial polymer molecular weight influences the degradation rate. Degradable polymers containing water-soluble polymer elements have been described. For example, Sawhney *et al.*, (1990) *J. Biomed. Mater. Res.* 24:1397-1411, copolymerized lactide, glycolide and  $\epsilon$ -caprolactone with PEG to increase its hydrophilicity and degradation rate. U.S. Patent No. 10 4,716,203 to Casey *et al.* (1987) also reports synthesis of PGA-PEG diblock copolymers, again with PEG ranging from 5-25%. Degradable materials of biological origin are well known, for example, crosslinked gelatin. Hyaluronic acid has been crosslinked and used as a degradable swelling polymer for biomedical applications (Della Valle *et al.* (1991) *Polym. Mater. Sci. Eng.*, 62:731-735]). U.S. Patent No. 5,410,016 issued April 25, 1995 describes biodegradable copolymers including water soluble polymers for use as photopolymerizable tissue coatings and controlled release vehicles.

*Materials to be coupled to or delivered via the Polymers*

Examples of bioactive materials which can be coupled to or 20 incorporated within the polymeric materials include proteins and peptides, sugars and polysaccharides, organic compounds with drug activity, nucleic acids, and combinations thereof, such as glycoproteins and drug conjugates. A peptide such as RGD, or even a single amino acid, which is used to target a polyamino acid for cleavage by an enzyme, can be incorporated into the 25 polymer structure, to direct attachment, or for subsequent polymer modification.

The bioactive, prophylactic, or diagnostic species can be attached to the copolymers, either covalently or ionically, or by mixing the species with the polymeric material, preferably before it is applied to tissue or used to 30 form a device or multilayer structure.

A wide variety of biologically active materials can be encapsulated or incorporated, including proteins such as antibodies, receptor ligands and

enzymes, peptides such as adhesion peptides, sugars, oligosaccharides, and polysaccharides, organic or inorganic drugs, nucleotides and nucleic acids, and cells, tissues or subcellular organelles or other subcellular components.

Exemplary ligands other than RGD include the pentapeptide Tyr-Ile-Gly-Ser-Arg (YIGSR), which supports endothelial, smooth muscle cell, and fibroblast adhesion, but not platelet adhesion; and the tetrapeptide Arg-Glu-Asp-Val (REDV), which has been shown to support endothelial cell adhesion but not that of smooth muscle cells, fibroblasts, or platelets, as described in Hubbell *et al.*, *BioTechnology* 9:568-572 (1991). YIGSR, from laminin, binds to receptors on endothelial cells, but not on blood platelets. Thus, application of a copolymer having conjugated thereto the peptide YIGSR to a damaged vessel wall would be expected to block thrombosis on the vessel wall but not to block reendothelialization from the surrounding undamaged vessel wall.

Exemplary diagnostic agents include diagnostic enzymes and radiolabelled and fluorescent compounds.

## II. Formation of Gels, Coatings, Devices and Multi-layer Structures

Boronic acid containing polymers are used to form bioinert gels and multilayer surface structures.

### 20 Formation of Gels and Coatings

The polymers form crosslinked hydrogels, which are highly swollen in water. The crosslinking can either be chemical or physical. Water soluble polymers containing boronic acid groups, such as phenylboronic acid (PBA), can be physically crosslinked by mixing the polymers in water with other polymers containing hydroxyls or carboxylic acids. As discussed below, multilayer structures are formed using alternating layers of boronic acid containing polymers and a diol or carboxylic acid containing polymer applied to each surface the boronic acid polymer is applied to, to provide sites for adhesion.

30 The boronate polymer can be applied in a fluid phase to the surface, such as tissues or cells, to be protected, whereupon the boronate groups adsorb the polymeric material to the surface. The fluid phase can be applied

to isolated tissue or to tissue during surgery or by means of a catheter or other less invasive device. Priming is generally not necessary in the case where the solution is applied to cells or tissue surface, because the boronic acid domains bind to diols present in glycosylated proteins present in the 5 cells.

Formation of Polymeric Multilayers

Multilayer techniques have been previously studied for surface modifications (G. Decher & J. Hong, Ber. Bunsenges. Phys. Chem. 95:1430-1434, 1991), and used to encapsulate living cells for transplantation (A. 10 Sawhney et al., Biomaterials, 13:863-870, 1992). Previous work has utilized polycations and polyanions, which also form gels when mixed in certain proportions under certain conditions.

The use of the boronic acid polymers described herein to form multilayers of polymers avoids some of the toxicity problems associated with 15 the polycation and polyanion materials. Surfaces can be treated by stepwise incubation with a solution of the boronic acid containing polymer, followed by incubation with a solution of a diol or carboxylic acid containing polymer to generate many successive layers, increasing the thickness of the formed structure at each step. Treatment of the surface is dependent upon the 20 surface activity of the boronate containing polymer or the diol or carboxylic acid containing polymer, or the use of a priming layer, consisting of a molecule which has an affinity for the surface, as well as an affinity for the boronate containing polymer or the diol or carboxylic acid containing polymer. These molecules do not interact via salt bridge formation, avoiding 25 the extreme toxicity of polycationic molecules when in solution (S. Choksakulnimitr et al., J. Contr. Rel., 34:233-241, 1995) which is an issue during application of the polycationic/polyanionic structure, and during degradation of the structure. The bioinertness of such materials may also be higher when at least one of the components is nonionic.

30 As noted above, the boronate polymer can be applied in a fluid phase to the surface, such as tissues or cells, to be protected, where the cells or tissue is in isolated form or during surgery or by means of a catheter or other

less invasive device. The diol or carboxylic acid polymer is then added in a fluid phase and crosslinks the already applied boronate polymer.

This process is repeated until the desired thickness is obtained. This process is referred to herein as "multilayer techniques". If only a monolayer 5 of each boronate containing polymer adsorbs with each incubation, then the coating can be built on a surface a few microns at a time. There are preferably greater than five alternating layers, more preferably more than ten alternating layers, and most preferably, greater than fifteen alternating layers of the polymers.

10 In another embodiment, the surface is not thoroughly rinsed between the application of the polymers. This leads to the formation of thicker structures. An apparatus equipped with a spray nozzle can be used, for example, to spray a layer at a time of a boronate containing polymer followed by a layer of a diol or carboxylic acid containing polymer.

15 Alternatively, both polymers can be sprayed simultaneously to create relatively thicker layers.

Thickness of the coating can be varied by selection of the reaction components and/or the reaction conditions. For example, the layer thickness can be controlled by adjusting the number of layers and also the degree of 20 rinsing between layers. Control of drop size and density during spraying of polymer yields coatings of the desired thickness without necessarily requiring rinsing between layers. Additionally, the excess (unbound) material can be removed via other means, for example, by an air jet. The polymer systems can be used to generate thick, non-adhesive films by 25 increasing the number of cycles, for example, to 50 or higher.

#### Implant or Device Coatings

The boronic acid polymer is preferably applied to a device having on its surface sites crosslinking with the boronic acid polymer. This can be achieved either by selection of a substrate with suitable functional groups, or 30 by application to the surface of a solution of a polymeric material containing diol and/or carboxyl groups. For example, a buffered solution of poly(acrylamide-co-acrylamidophenylboronic acid) can be incubated with a

porcine valve implant, followed by washing with buffered saline. The implant can then be incubated with a buffered solution of hyaluronic acid, dextran, or polysialic acid, followed by washing. This process could be repeated five or more times to produce a bioinert surface coating on the 5 implant, of varying thickness depending upon the number of repetitions.

### III. Methods of Using the Compositions

Multilayers of the polymers can be formed on macroscopic tissue surfaces, including mammalian tissue surfaces, and thereby provide various benefits to the coated surfaces. These include the prevention of adherence of 10 tissue to tissue, or of cells to tissue, or provision of selective adherence, as described below. The layers can be used to encapsulate, plug, seal, or support a macroscopic surface. The application of a multilayer coating can be used to minimize or prevent tissue adhesion, minimize or prevent postoperative adhesions, prevent thrombosis, prevent implantation of 15 cancerous cells, coat tissue to encourage healing or prevent infection, or enhance the local delivery of bioactive agents. Preferably, at least four layers, and, more preferably, at least six layers, are used to form the coatings.

The compositions, while serving as bioinert scaffolds, can also serve to provide biospecific signaling.

20 The same type of technique can be used to coat the inside of an artery following balloon angioplasty, to reduce thrombosis; to coat the surface of red blood cells to enhance the efficacy of blood transfusion; to coat a tissue engineered implant, and to coat a tissue which has been damaged during surgery.

We claim:

1. A coating on the surface of cells, tissue, or a device for implantation into a patient comprising a boronic acid polymer crosslinked with a diol or carboxylic acid containing polymer or substrate.
2. The coating of claim 1, comprising alternating layers of the boronic acid containing polymer and a diol or carboxylic acid containing polymer.
3. The coating of claim 1 wherein the diol or carboxylic acid containing polymer is a water soluble polymer.
4. The coating of claim 1 wherein the diol or carboxylic acid containing substrate is the surface of the cells or tissue.
5. The coating of claim 1 wherein the substrate is the surface of a medical device.
6. The coating of claim 2 comprising at least five layers of polymer.
7. The coating of claim 1 further comprising an agent selected from the group consisting of therapeutic, prophylactic or diagnostic agents, coupled to or incorporated in, the polymer.
8. The coating of claim 1 wherein the boronic acid polymer comprising functional groups or domains modifying the polymer properties.
9. The coating of claim 8 wherein the polymer comprises biodegradable groups or domains.
10. A method for coating, encapsulating, plugging, sealing, or supporting a surface, comprising depositing a boronic acid polymer solution onto the surface, wherein the surface comprises diol or carboxylic acid groups which crosslink with the boronic acid polymer.
11. The method of claim 10 further comprising coating the surface with a diol or carboxylic acid containing polymer prior to depositing the boronic acid polymer solution thereon.
12. The method of claim 10 further comprising applying to the boronic acid polymer a layer of a diol or carboxylic acid containing polymer to form a multi-layer structure.

13. The method of claim 12 further comprising alternately repeating the steps of depositing the boronic acid containing polymer and applying the diol or carboxylic acid containing polymer to form additional layers of the multi-layer structure.

14. The method of claim 10, wherein the boronic acid polymer is applied to cell or tissue surfaces to minimize or prevent tissue adhesion.

15. The method of claim 14 wherein the polymer is applied postoperatively, or during angioplasty, to minimize or prevent postoperative adhesion, thrombosis, or to encourage healing or prevent infection.

16. The method of claim 14 wherein the polymer has coupled to or incorporated therein an agent selected from the group consisting of therapeutic, diagnostic or prophylactic agents, comprising applying the polymer to a site for delivery of the agent.

17. The method of claim 10 wherein the polymer is applied to the surface of a medical device.

18. The method of claim 10 comprising applying the polymer to cancer cells to seal and encapsulate the seals to minimize metatasis of the cells.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/19633

**A. CLASSIFICATION OF SUBJECT MATTER**  
**IPC 7 A61L31/08 A61L27/28**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

**IPC 7 A61L**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p><b>DATABASE WPI</b>  <b>Section Ch, Week 199509</b>  <b>Derwent Publications Ltd., London, GB;</b>  <b>Class A89, AN 1995-063344</b>  <b>XP002124863</b>  <b>&amp; JP 06 339367 A (NIPPON OILS &amp; FATS CO LTD), 13 December 1994 (1994-12-13)</b>  <b>abstract</b></p> <hr/> <p><b>AOKI T ET AL: "Endothelial cell differentiation into capillary structures by copolymer surfaces with phenylboronic acid groups"</b>  <b>JOURNAL OF BIOMATERIALS SCIENCE. POLYMER EDITION, NL, VSP, UTRECHT,</b>  <b>vol. 7, no. 7, page 539-550 XP002097651</b>  <b>ISSN: 0920-5063</b>  <b>abstract</b></p> <hr/> <p style="text-align: center;">-/-</p>	1-18
X		1-9

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- \*P\* document published prior to the International filing date but later than the priority date claimed

- \*T\* later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- \*&\* document member of the same patent family

Date of the actual completion of the International search

6 December 1999

Date of mailing of the International search report

17/12/1999

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ESPTNOSA M

## INTERNATIONAL SEARCH REPORT

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 99 10022 A (CALIFORNIA INST OF TECHN) 4 March 1999 (1999-03-04) the whole document	1-18
A	HUBBELL J A: "Hydrogel systems for barriers and local drug delivery in the control of wound healing" JOURNAL OF CONTROLLED RELEASE, NL, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, vol. 39, no. 2, page 305-313 XP004037336 ISSN: 0168-3659 abstract	1-18
A	SAWHNEY A S ET AL: "INTERFACIAL PHOTOPOLYMERIZATION OF POLY(ETHYLENE GLYCOL)-BASED HYDROGELS UPON ALGINATE-POLY(1-LYSINE) MICROCAPSULES FOR ENHANCED BIOCOMPATIBILITY" BIOMATERIALS, GB, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, vol. 14, no. 13, page 1008-1016 XP000404244 ISSN: 0142-9612 abstract	1-18
A	HAN D K ET AL: "LACTIDE-BASED POLY(ETHYLENE GLYCOL) POLYMER NETWORKS FOR SCAFFOLDS IN TISSUE ENGINEERING" MACROMOLECULES, US, AMERICAN CHEMICAL SOCIETY. EASTON, vol. 29, no. 15, page 5233-5235 XP000596745 ISSN: 0024-9297 the whole document	1-18
A	HAN D K ET AL: "SYNTHESIS OF POLYMER NETWORK SCAFFOLDS FROM L-LACTIDE AND POLY(ETHYLENE GLYCOL) AND THEIR INTERACTION WITH CELLS" MACROMOLECULES, US, AMERICAN CHEMICAL SOCIETY. EASTON, vol. 30, no. 20, page 6077-6083 XP000702263 ISSN: 0024-9297 abstract	1-18
A	US 5 647 858 A (DAVIDSON JAMES A) 15 July 1997 (1997-07-15) column 3, line 15	1
A	US 5 410 016 A (HUBBELL JEFFREY A ET AL) 25 April 1995 (1995-04-25) cited in the application claims; examples	1

**INTERNATIONAL SEARCH REPORT**International application No.  
PCT/US 99/19633**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: —  
because they relate to subject matter not required to be searched by this Authority, namely:  
**Although claims 14-16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.**
2.  Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claim No.:

**Remark on Protest**

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/19633

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